

CLAIMS:

1. A protein separation device comprising a chaperone protein immobilised on a substrate.
2. The protein separation device as claimed in claim 1, in which the chaperone protein is selected from the group consisting of Hsp100, Hsp90, Hsp70, Hsp60 and small Hsps.
3. The protein separation device as claimed in claim 2, in which the Hsp60 chaperone is a group one chaperone.
4. The protein separation device as claimed in claim 3, in which the group one chaperone is GroEL.
5. The protein separation device as claimed in claim 4, in which GroEL is in a single ring configuration.
6. The protein separation device as claimed in any one of claims 4 to 7, in which GroEL comprises a back-to-back double ring configuration, wherein one ring is heptameric and the other ring is dimeric, trimeric, tetrameric, pentameric or hexameric.
7. The protein separation device as claimed in any one of claims 4 to 7, in which GroEL comprises an heptameric ring of wild-type GroEL and a ring of another chaperonin protein.
8. The protein separation device as claimed in claim 7, in which the other chaperonin protein is rubisco subunit binding protein or CCT.
9. The protein separation device as claimed in any one of claims 4 to 8, in which GroEL comprises a double ring assembly wherein one or both rings comprise one or more subunits from other chaperonins.

10. The protein separation device as claimed in claim 9, in which each ring contains one or more of the α , β , γ , δ , ϵ , ζ or θ subunits of CCT.
11. The protein separation device as claimed in claim 9, in which each ring contains one or more subunits of rubisco subunit binding protein.
12. The protein separation device as claimed in any one of claims 9 to 11, in which each ring is a heteromeric heptamer.
13. The protein separation device as claimed in claim 4 to 12, in which GroEL is in operative association with a co-factor.
14. The protein separation device as claimed in claim 13, in which the co-factor is GroES.
15. The protein separation device as claimed in any one of claims 1 to 14, in which the chaperone is obtainable from a microbial source selected from bacteria and archaea.
16. The protein separation device as claimed in claim 15, in which the microbial source is selected from the group consisting of *Escherichia spp.*, *Thermus spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, *Bacillus spp.*, *Leptospira spp.*, *Spirillum spp.*, *Lactobacillus spp.*, *Mycoplasma spp.*, *Pseudomonas spp.*, *Streptomyces spp.*, *Corynebacterium spp.*, *Bacteroides spp.* and *Clostridium spp.*
17. The protein separation device as claimed in claim 15, in which the *Escherichia spp.* microbial source is *Escherichia coli*.

18. The protein separation device as claimed in claim 15, in which the *Thermus spp.* microbial source is *Thermus thermophilus*.
19. The protein separation device as claimed in claim 5 15, in which the *Clostridium spp.* microbial source is *Clostridium difficile*.
20. The protein separation device as claimed in any one of claims 1 to 19, in which the substrate is a solid support of the array or bead type.
- 10 21. The protein separation device as claimed in claim 20, in which the substrate is manufactured from a plastics material.
22. The protein separation device as claimed in claim 20 or 21, in which the support of the array type is provided 15 with a surface for immobilisation of a protein of the chaperone type thereon.
23. The protein separation device as claimed in claim 22, in which the surface is comprised of moieties selected from the group consisting of nitriloacetic acid, avidin, 20 streptavidin, carboxylates, quaternary amines, silicates, carbonyl diimidazoles and epoxides.
24. The protein separation device as claimed in claim 23, in which the surface is provided with an hydrophobic barrier coating.
- 25 25. The protein separation device as claimed in any one of claims 1 to 25, in which the biological sample is selected from the group consisting of cerebrospinal fluid, urine, nipple aspirant, other biological fluids and extracts.

26. The protein separation device as claimed in any one of claims 1 to 25, in which the biological sample is denatured.

27. The protein separation device as claimed in claim 5 26, in which the biological sample is denatured by at least one denaturant selected from the group consisting of chaotropic agents, detergents, heat and reducing agents.

28. The protein separation device as claimed in claim 10 27, in which the chaotropic agent is be selected from guanidine hydrochloride, guanidine thiocyanate, urea, thiourea, sodium thiocyanate and ammonium sulphate.

29. The protein separation device as claimed in claim 27, in which the detergent is sodium dodecyl sulphate.

30. The protein separation device as claimed in claim 15 27, in which the reducing agent is selected from the group consisting of dithiothreitol (Cleland's reagent), dithioerythritol and 2-mercaptoethanol.

31. The protein separation device as claimed in any one of claims 1 to 26, in which the biological sample is 20 denatured by a combination of any one of the denaturing agents as claimed in claim 37.

32. A protein separation device comprising GroEL immobilised on a substrate in an optimised orientation to bind a target protein and to provide minimal steric 25 hindrance between GroEL and the substrate.

33. A protein separation device as claimed in claim 32, in which GroEL is engineered by site-directed mutagenesis to substitute Aspartate490 with a cysteine.

34. A protein separation device as claimed in claim 33, in which the substituted cysteine is reacted with biotin to form a biotin-cysteine conjugate.

35. A protein separation device as claimed in claim 5 34, in which the biotin-cysteine conjugate is reactable with an avidin or streptavidin moiety located on a surface of a substrate.

36. A protein separation device comprising GroEL immobilised on a substrate, wherein the specificity of GroEL 10 is directed to a particular protein.

37. A protein separation device as claimed in claim 36, in which GroEL is engineered by site-directed mutagenesis to have the substitutions, leucine 200 to arginine; serine 201 to glycine, and proline 202 to 15 aspartate.

38. A protein separation device as claimed in claim 37, in which the substitutions introduce an integrin binding motif into a protein binding domain of GroEL.

39. A protein separation device comprising GroEL 20 immobilised on a substrate wherein the specificity of GroEL is changed to a protein specificity of another chaperone protein.

40. A protein separation device as claimed in claim 39, in which GroEL is engineered by site-directed 25 mutagenesis to have the substitutions, Tyrosine 199 to Isoleucine; Tyrosine 204 to Isoleucine; Leucine 234 to Isoleucine; Leucine 237 to Isoleucine; Leucine 259 to Phenylalanine; Valine 263 to Leucine and Valine 264 to Phenylalanine.

41. A protein separation device as claimed in claim 39 or claim 40, in which the substitutions are made in the apical protein-binding domain of GroEL.

42. A protein separation device as claimed in claim 40
5 or claim 41, in which the substitutions replace the substrate binding specificity of GroEL, a group I chaperone, with that of thermosome, a group II chaperone.

43. A protein separation device comprising GroEL
10 arranged to have an optimised orientation to bind a target protein and minimal steric hindrance between GroEL and the substrate when GroEL is immobilised on the substrate and its specificity for a target protein is directed to a particular target protein.

44. A protein separation device comprising GroEL in an
15 optimised orientation to bind a target protein and to provide minimal steric hindrance between GroEL and the substrate wherein the specificity of GroEL for a target protein is changed to a specificity of another chaperone protein.

20 45. A method of isolating at least one protein from a biological sample comprising the steps of:

- a) denaturing a biological sample containing at least one protein;
- b) applying the biological sample containing at the least one
25 protein to a chaperone protein immobilised on a substrate.
- c) isolating the at least one protein from the biological fluid on the chaperone protein;

- d) removing the biological sample from the chaperone protein immobilised on the substrate, and
- e) obtaining the at least one protein from the chaperone protein.

5 46. A method of identifying a biological marker in a biological sample comprising the steps of:

- a) applying the biological sample containing the biological marker to a chaperone protein immobilised on a substrate;
- b) isolating the biological marker from the biological fluid
10 on the chaperone protein;
- c) removing the biological sample from the chaperone protein, and
- d) obtaining the at least one protein from the chaperone protein immobilised on the substrate.

15 47. A method of diagnosis comprising the steps of:

- a) applying a biological sample from a first subject to a chaperone protein immobilised on a substrate;
- b) isolating a protein from the biological fluid on the chaperone protein;
- 20 c) removing the biological sample from the chaperone protein;
- d) obtaining the at least one protein from the chaperone protein, and
- e) Comparing the concentration of the at least one protein from the first subject with a reference concentration
25 obtained from a second subject.

48. A method as claimed in claim 46 or 47 in which the biological sample is denatured prior to step a) of each method.

49. A method as claimed in any one of claims 45 to 48
5 in which the chaperone immobilised on a substrate comprises the protein separation device as claimed in any one of claims 1 to 44.